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PHYLOGENY OF *FRAXINUS* SECT. *MELIOIDES* (OLEACEAE): REVIEW AND AN ALTERNATIVE HYPOTHESIS

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ABSTRACT

Molecular-phylogenetic hypotheses for the species of *Fraxinus* sect. *Melioides* by Jeandroz (1997), Wallander (2008), and Hinsinger et al. (2013) are summarized and reviewed. There is disagreement among them and unexpected arrangements suggest that either misidentifications or speciation by hybridization, or both, may have affected the conclusions. An alternative phylogenetic hypothesis, subjectively developed mostly on the basis of morphology and geography, is presented here to summarize points of agreement and what seems plausible, perhaps to serve as a guide in further investigations. Sect *Melioides* is recognized here as comprising fifteen species divided informally among five groups: the Americana group (5 species), the Pennsylvanica group (7 species), and the Uhdei, Latifolia, and Papillosa groups (each with 1 species). Molecular data indicate that the American *F. cuspidata* and the Asian *F. platypoda*, *F. chiisanensis*, and *F. spaethiana* are closely related to sect. *Melioides* — these 4 species are regarded here as "aff. sect. *Melioides*," perhaps warranting separate formal recognition, since, if added to sect. *Melioides*, its morphological integrity is weakened.

Recent research by Hinsinger et al. (2013) is primarily concerned with patterns of diversification and geographic speciation in *Fraxinus* and provides interpretations of the geologic history of intercontinental dispersal and vicariance. They used "nuclear external transcribed spacers (nETS), phantastica gene sequences, and two chloroplast loci (trnH-psbA and rpl32-trnL) in combination with previously published and newly obtained nITS sequences to produce a time-calibrated multi-locus phylogeny of the genus." The phylogeny of *Fraxinus* also has been recently analyzed by Jeandroz et al. (1997, using nrITS) and by Wallander (2008, using nrITS). The Jeandroz study included 8 species of sect. *Melioides*, that of Wallander 10 species, that of Hinsinger et al. 9 species. Wallander and Hinsinger et al. included *F. cuspidata* of the western USA and three Asian species that molecular data indicate are closely related to sect. *Melioides*. None of the three studies appears to have included *F. smallii* (the tetraploid form of *F. americana*) or *F. pauciflora* — at least there were no samples identified as such.

Insofar as these three studies address broad patterns of diversification and biogeographic history and are relatively more reliant on identifications essentially at sectional level (rather than of individual species), results are plausible. But because of seeming anomalies in the relative phyletic positions of the sect. *Melioides* species, botanists interested in relationships among the North American *Fraxinus* species (which mostly are within sect. *Melioides*) probably will not be discouraged from further investigation. Hinsinger et al. (2013) suggested that apparent low differentiation at the genetic level among species of sect. *Melioides* (as interpreted from the poor phylogenetic resolution among samples) is a result of rapid radiation or recent gene exchange — explicitly eliminating misidentifications as a possible explanation (see below). Wallander (2008) noted that polyploidization and hybridization in sect. *Melioides* may be responsible for inconsistencies in the phylogenetic analysis; she also observed that "morphological and ecological variation" make identifications difficult.

Wallander (2008, p. 37) provided the following diagnostic description of sect. *Melioides*: "They are all medium-sized to large trees, deciduous and dioecious. The unisexual flowers are

apetalous and wind-pollinated. The female flowers consist of a calyx and one pistil, and the male flowers of two stamens with elongated anthers and a small calyx. There are no rudimental organs of the opposite sex in the flowers (a unique synapomorphy for this section). The calyx is persistent in the samaras, which have a distinctly terete seed cavity (except *Fraxinus caroliniana*). The wing may be decurrent along the seed cavity or not. In addition, the presence of flavones in the leaves (besides the plesiomorphic flavonols) is a synapomorphy for these species." While flavones are consistent feature of the the North American species, they also occur in *F. chiisanensis* (Chang et al. 2002), one of the species closely allied to sect. *Melioides* by molecular data, and Lee et al. (2012) found that they occur sporadically in Asian species of sect. *Ornus*.

Table 1. ***Fraxinus*** **sect.** ***Melioides***

Sect. Melioides

Pennsylvanica Group
Fraxinus profunda
Fraxinus pennsylvanica
Fraxinus berlandieriana
Fraxinus velutina
Fraxinus coriacea
Fraxinus caroliniana
Fraxinus cubensis

Latifolia Group
Fraxinus latifolia

Papillosa Group
Fraxinus papillosa

Uhdei Group
Fraxinus uhdei

Americana Group
Fraxinus americana
Fraxinus albicans (syn: F. texensis)
Fraxinus smallii
Fraxinus biltmoreana
Fraxinus pauciflora

Aff. sect. Melioides

Asian Group
Fraxinus chiisanensis
Fraxinus platypoda
Fraxinus spaethiana

Cuspidata Group
Fraxinus cuspidata

In the taxonomic perspective here, *Fraxinus* sect. *Melioides* includes 15 North American species (Table 1). Molecular data indicate that (a) three species from southeast Asia and (b) one from the southwestern USA are most closely related to sect. *Melioides* — Hinsinger et al. explicitly included them as members of the section while Wallander mostly treated them as *incertae sedis* in her classification, as morphology places them outside of traditional sect. *Melioides*.

(a) In the Hinsinger et al. analysis *F. chiisanensis* (Korea), *F. platypoda* (China), and *F. spaethiana* (Japan) are members of the same clade and this small group is sister to North American sect. *Melioides* (Fig. 2). *Fraxinus platypoda* was treated by Wallander (2008) within sect. *Fraxinus* while she placed *F. chiisanensis* and *F. spaethiana* as *incertae sedis*; these three Asian species differ from North American sect. *Melioides* in their polygamous sexual condition (vs. strict dioecy in the North American species) and flavonoid chemistry (Lee et al. 2012). Wallander reviewed other evidence (mainly floral morphology and leaf flavonoids) relative to the phylogenetic position of these species. In a formal classification, these three could reasonably be treated either as a separate section or as a part of sect. *Melioides*.

(b) The American *F. cuspidata* was regarded by Jeandroz et al. as a member sect. *Dipetalae,* by Wallander as "incertae sedis." A consensus tree in the Jeandroz et al. analysis (their Figure 4, excerpted here as Figure 3) positioned *F. cuspidata* as sister to North American sect. *Melioides*. The Wallander analysis (sect. *Melioides* excerpted here as Figure 4) positioned *F. cuspidata* as sister to *F. spaethiana*, within the same clade of three Asian species as identified by Hinsinger et al. In the Hinsinger et al. analysis (their Figure 2, and as represented here in Figure 1), however, *F. cuspidata* is sister to the North American/Asian pair of clades. Following Wallander's suggestion, a case could be

made for treating *F. cuspidata* as a monotypic section, based on its apparently isolated phylogenetic position and distinctive morphology (flowers bisexual and anemophilous; petals 4, white, united in a tube 2–3 mm, lobes linear, 5–6 mm).

Within sect. *Melioides*, five informal groups are recognized here (Table 1). Among the eastern and southwestern North American species, the Americana Group (the white ash group) is highly distinctive in morphology, with the abaxial leaf surfaces completely covered by a waxy reticulum — this structure is viewed here as a single-origin feature indicating that at least the genome underlying this epidermal expression in the Americana Group is monophyletic. In all of the other species, the epidermis of the abaxial leaf surface is exposed, without wax, as also in all other species of the genus. *Fraxinus uhdei* (Mexico and Central America) and *F. latifolia* (Pacific coast region) are set apart here as the Uhdei Group and the Latifolia Group because of their geographic disjunctions and because there seems to be agreement among the molecular analyses (Figs. 2, 3, 4) that neither species arose from within the Pennsylvanica Group as recognized here, which essentially comprises the remainder of the species.

Among the three published phylogenetic hypotheses for sect. *Melioides*, the most unexpected species placements reflect an apparent lack of coherence among the species of the Americana Group — their dispersal among species of other groups suggests that either misidentifications or speciation by hybridization, or both, are reflected in the cladogram topologies. An alternative hypothesis, subjectively developed mostly on the basis of morphology and geography, and with attention to consistencies in the molecular analyses, is presented here (Fig. 1) to summarize what seems plausible and perhaps to serve as a guide or model in further investigations.

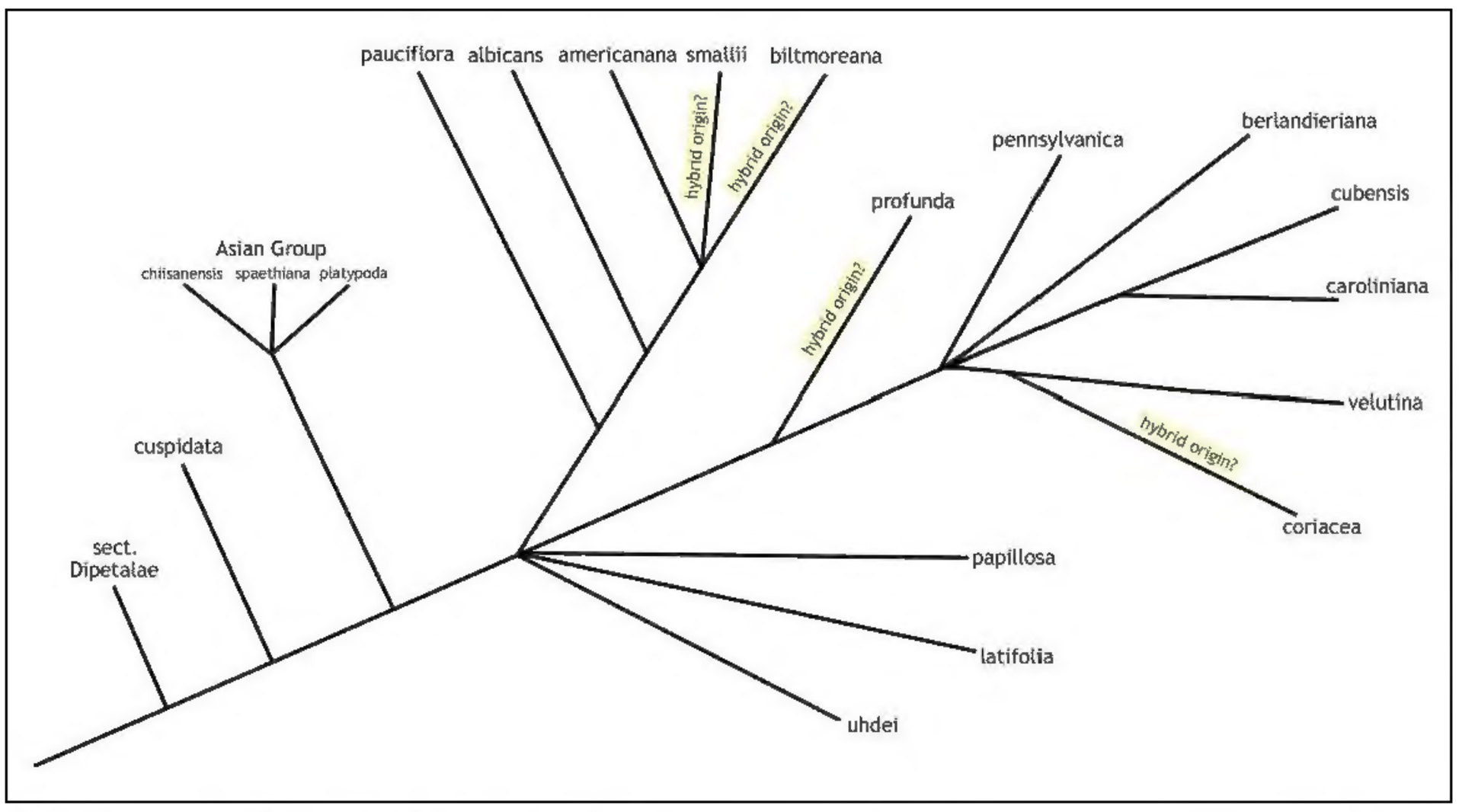

Figure 1. Hypothetical, subjectively formulated phylogeny, based on morphology and geography, of sect. *Melioides*. *Fraxinus cuspidata* and the Asian group are generally indicated by the molecular phylogenies to have a sister or basal relationship to the traditionally recognized North American species of the section. The position of sect. *Dipetalae* as basal in the whole genus is indicated by both Wallander and Hinsinger et al. *Fraxinus smallii* (tetraploid), *F. biltmoreana* (hexaploid), and *F. profunda* (hexaploid), and perhaps *F. coriacea* (tetraploid) may be of hybrid origin.

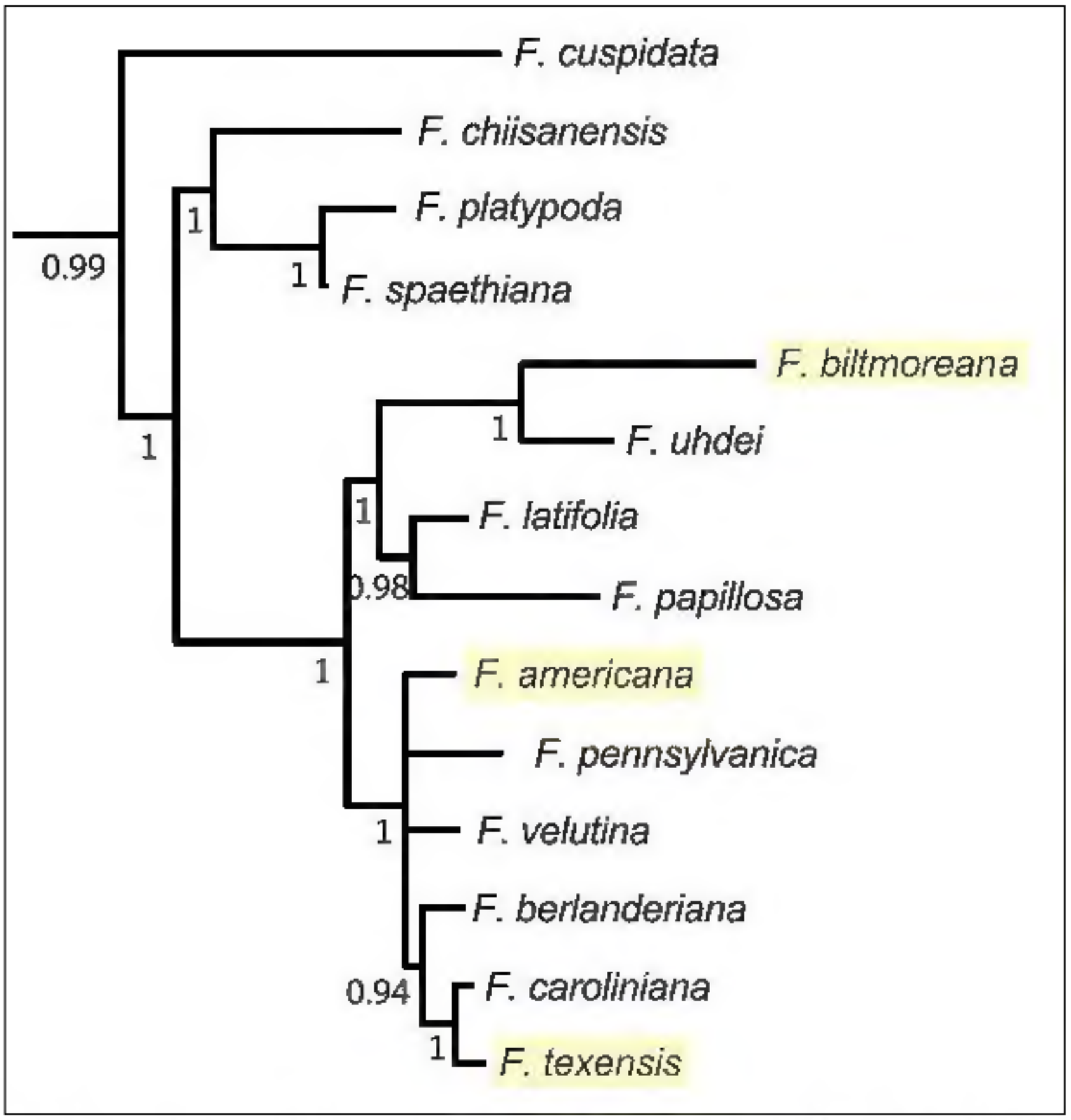

Figure 2. Phylogeny of sect. *Melioides*, extracted from Figure 1 of Hinsinger et al. (2013), which shows a Bayesian analysis of the combined dataset (cpDNA, ETS, ITS and phantastica). Posterior probabilities ≥0.50 are indicated below the branches. The yellow-highlighted samples of *F. americana* and *F. texensis* appear to be out of place within the clade that otherwise comprises red ash species — based on morphology they would be expected to cluster with *F. biltmoreana* (also yellow-highlighted). In any case, apart from *F. latifolia* and *F. uhdei*, the subjective and arbitrary sample selection involved in the derivation of this tree appears to render it essentially meaningless (see text).

Identifications

In recent studies of *Fraxinus*, I found that a significant proportion of herbarium specimens of North American *Fraxinus* have been misidentified, even in collections amassed or curated by experienced taxonomists. This is especially true for sect. *Melioides*, where *F. americana* and *F. pennsylvanica* commonly are confused. While this might suggest that taxonomic distinctions can be subtle, it does not indicate that the species are indistinct. Implicit here is the notion that species boundaries can be discerned among the ash species, and having intensively studied the North American taxa (Nesom 2010a-h; Nesom submitted; Williams & Nesom 2010), I believe this is possible, even though some of the species are variable. A possible exception to this has been in the attempt to understand the pattern of variation in the *F. velutina-papillosa* complex — *F. papillosa* is re-segregated here, based in part on reconsideration of molecular evidence (see comments below).

The 57 North American sect. *Melioides* samples of Hinsinger et al. were mostly from arboreta (mostly European, some in the USA); in two species (*F. berlandieriana*, *F. papillosa*) sampling was augmented by herbarium specimens (total of 5) from MEXU. Of the 52 total arboretum collections, 33 were vouchered (specimens deposited at P). By sampling multiple individuals for each taxon, they "expected that, if hybridization or misidentification occurred, only one or a few individuals in a particular species would be affected; as a consequence, most individuals would still be classified in a monophyletic group representative of the species." Such did not prove to be the case for North American sect. *Melioides*, however, as inconsistency of phyletic position for samples within a single taxon was a significant feature of the analyses of individual data sets where all samples are shown (the maximum likelihood analyses). It seems a reasonable guess that arboretum identifications were accepted at face value in the Hinsinger et al. study, since who among the authors might have provided confirmations of identity was not indicated.

The maximum likelihood (ML) analyses of Hinsinger et al. include all of their samples (see Figs. S2, S3, S4 in their Supporting Information). Their Baysian (BIM) analyses, however, including that of the combined dataset shown in their Figure 1 (Fig. 2 above), were done with a reduced dataset. "All BIM analyses were performed on a reduced dataset using MrBayes. This reduced dataset was created by using one individual per species following Wallander, with the selection of individuals located within **the ML tree** [emphasis added] at positions that were reasonable considering their identity for each data partition, that is, grouping with other individuals from the same species" (pp. 4–5). Presumably they meant "the ML trees" (S2, S3, S4). But whether they used a single (unspecified) ML tree or all three, their process of dataset reduction for sect. *Melioides* appears to have been almost entirely subjective. Positions on the S4 tree seem utterly random for all samples. On the S2 and S3 trees, the only species that cluster unambiguously are *F. latifolia* and *F. uhdei* — for species where half or more than half of the samples are clustered and the single sample was selected from the largest cluster, *F. velutina* is the only candidate. All other choices appear arbitrary.

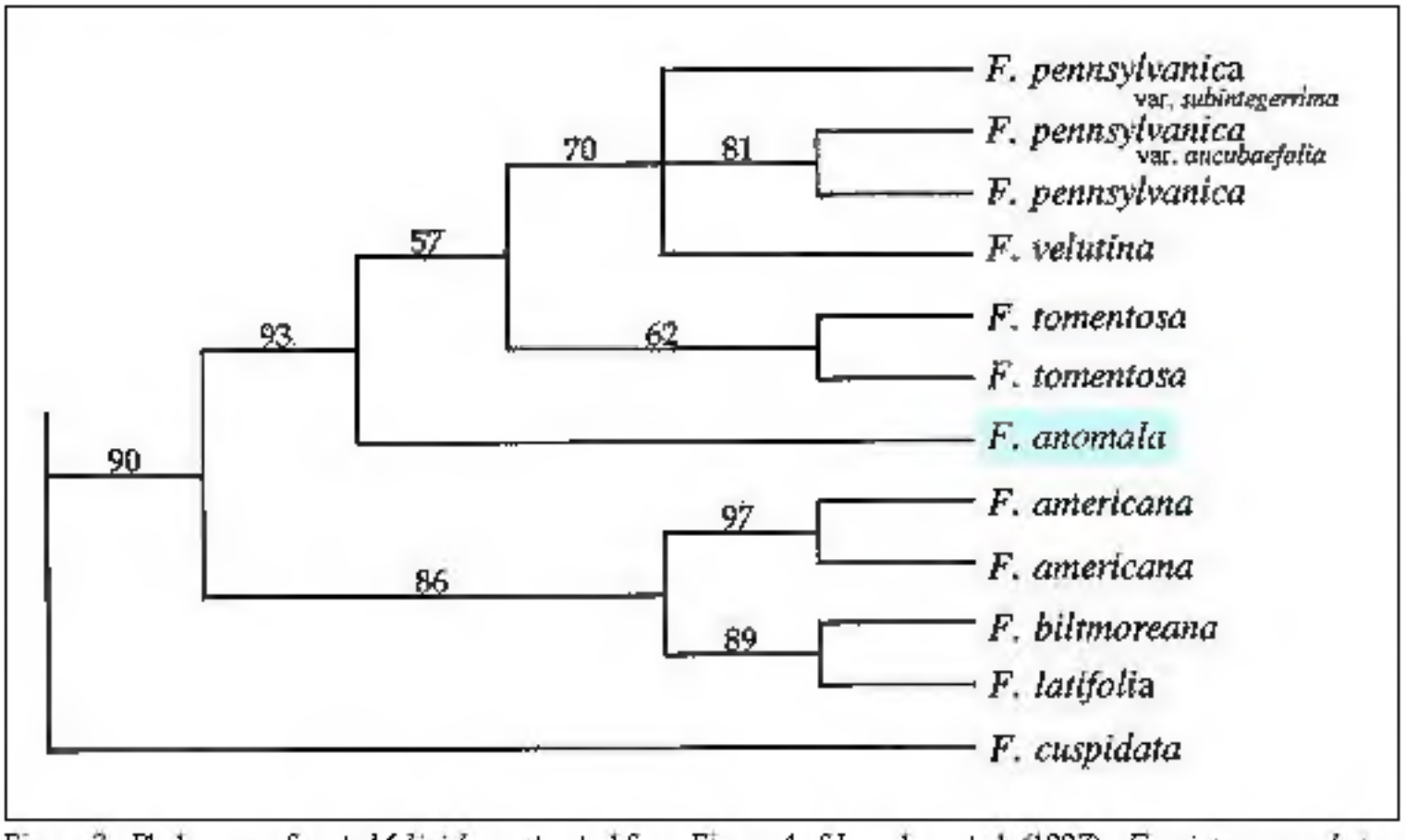

Figure 3. Phylogeny of sect. *Melioides*, extracted from Figure 4 of Jeandroz et al. (1997). *Fraxinus anomala* is clearly a member of sect. *Dipetalae* and probably appears within the topology of sect. *Melioides* a result of misidentification.

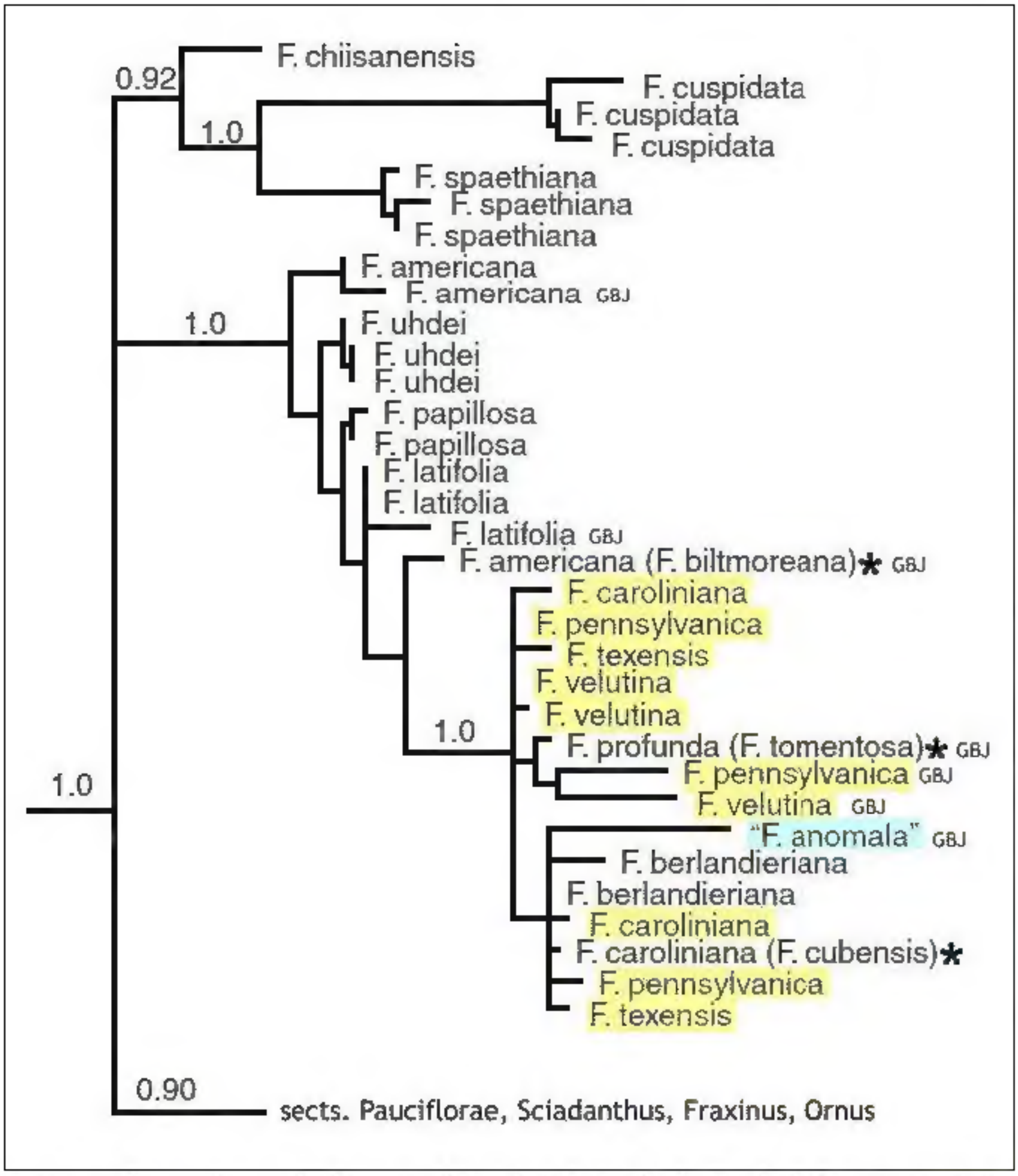

Figure 4. Phylogeny of sect. *Melioides*, extracted from Figure 1 of Wallander (2008), which portrays a majority rule consensus tree resulting from the Bayesian analysis of 111 ITS sequences representing 40 Fraxinus species and five Oleaceae outgroup species. Bayesian posterior probabilities are shown above the branches. Starred samples are species represented by only one sample. Yellow-highlighted samples represent species placed in positions separated from one another, probably indicating either hybridization or misidentification. "GBJ" indicates a GenBank sequence originally used in the Jeandroz et al. analysis. The "F. anomala" sample almost certainly is misidentified, as recognized by Wallander.

All of the sect. *Melioides* samples in the Jeandroz et al. analysis are noted as having originated from the Montreal Botanic Garden. Vouchers apparently were not made for those samples or any others in the study.

Wallander's samples of sect. *Melioides* were from cultivated trees at arboreta (Missouri Botanical Garden, Kew Garden, vouchers at GB), field collections from Oregon and Washington (*F. latifolia*, vouchers at GB), herbarium specimens (GB, MO, NY, S), and GenBank data (1 sample of *F. pennsylvanica*, 1 of *F. profunda*, 1 of *F. velutina*).

The Americana group

The Americana Group is distinctive in the waxy reticulum overlying the abaxial leaf surface, a feature without parallel elsewhere in the genus (or, apparently, elsewhere in the family).

* *Fraxinus albicans* (= *F. texensis*; chromosome number unreported) is closely similar to diploid *F. americana* — the main differences are in leaf and fruit size and geography (Nesom 2010b). If *F. albicans* proves to be diploid, without hybrid origin, then it is unexpected that *F. albicans* should be placed apart from *F. americana* in the Wallander analysis (Fig. 4) and, further, that its two samples should be in separated positions. In any case, a duplicate of the voucher for "texensis2" (TEX; from Coryell Co., Texas; in the "upper" position in Fig. 4) indeed is correctly identified.

* *Fraxinus smallii* (tetraploid) and *F. biltmoreana* (hexaploid) presumably include the genome of diploid *F. americana* and it is reasonable to speculate that their extra chromosome sets were acquired via hybridization with forms of *F. pennsylvanica* or some other species of green ash. Such interspecific hybridization might account for the seemingly disparate phylogenetic positions of these species in molecular analyses. Or the ancestry of *F. smallii* and *F. biltmoreana* may have involved species of the Americana Group no longer extant. *Fraxinus smallii* differs from *F. americana* mostly in fruit size and petiole shape and the former may have originated as an autopolyploid or segmental allopolyploid. No data exists at present to support any hypothesis of reticulate evolution in sect. *Melioides*.

* *Fraxinus pauciflora* (Nesom 2010c) is distinct in geography, ecology, and morphology and is placed here (Fig. 4) as sister to the other white ash species — its chromosome number is unreported.

The Pennsylvanica Group

The Pennsylvanica Group is shown as monophyletic in Figure 4, but a clear morphological synapomorphy is not evident. The group is spread over eastern North America and central and southwestern states of the USA.

* *Fraxinus profunda* is distinct in its extremely large fruits and leaves, deep swamp ecology, and hexaploid constitution (Nesom 2010h) — it may be of hybrid origin, similar to the possibilities for *F. smallii* and *F. biltmoreana*.

* *Fraxinus pennsylvanica* is variable over its wide range and numerous segregates have been named, but a clear concept of morpho-geographic subentities has never been documented.

* *Fraxinus velutina* (Nesom 2010f; Williams & Nesom 2010) also is variable over a wide range. It apparently intergrades with *F. papillosa*, but this needs to be studied in detail.

* *Fraxinus coriacea* sometimes has been considered conspecific with *F. velutina* but is geographically and morphologically distinct (Nesom 2010e). Taylor (1945) reported a tetraploid chromosome count for *F. coriacea* (as "F. velutina var. coriacea"), in contrast to four diploid collections of *F. velutina* (reported as "F. velutina," "F. velutina var. glabra," and "F. velutina var. toumeyi").

* *Fraxinus caroliniana* and *F. cubensis* apparently are a sister pair with contiguous geographic ranges (Nesom 2010c). One of the two samples of *F. caroliniana* ("caroliniana1," from Florida) in Wallander's analysis is positioned close to *F. cubensis* but the other ("caroliniana2," from North Carolina) is separated (see Fig. 4). The "caroliniana2" sample (voucher at MO) is confirmed here as correctly identified as *F. caroliniana*.

* *Fraxinus berlanderiana* has sometimes been synonymized with *F. pennsylvanica* and perhaps is most closely related to it (Nesom 2010f). The tendency of *F. berlanderiana* to produce 3-winged fruits also is characteristic of *F. caroliniana/cubensis* but morphological dissimilarities suggest that this probably is a parallelism. One of Wallander's samples of *F. berlanderiana* was from Texas (*Jones 3595*, NY), the other from Hidalgo, Mexico (*Pringle 13584*, S) — the type of *F. pringlei* (*Pringle 9417*, valley near Dublan, Hidalgo) is from a tree similar to *Pringle 13584*. *Fraxinus pringlei* probably is justifiably treated as conspecific with *F. berlandieriana* (contrary to a prior assertion of mine; Nesom 2010f), but this needs study.

Monotypic species groups

Fraxinus papillosa. This has sometimes been compared with *F. americana* (see review comments in Williams and Nesom 2010) but it was tentatively considered a regional expression of *F. velutina* (Williams & Nesom; Nesom 2010). The whitened abaxial leaf surface of *F. papillosa* does not have the reticulate overlay of wax like that of the Americana group — each abaxial epidermal cell of *F. papillosa* is abruptly convex and formed upward into a short-cylindric, papilla-like structure (see SEMs in Williams and Nesom) with no obvious evidence of deposition of white-colored material. The white color perhaps due to increased reflectivity of the raised cells. Typical *F. velutina*, which has abaxial epidermal cells with green, relatively flat surfaces, appears to intergrade with *F. papillosa*, but the difference in morphological extremes is so great that the two probably would better be regarded as separate but intergrading species.

The molecular studies of Wallander (2008) and Hinsinger et al. (2013), based on independent sampling, suggest that *Fraxinus papillosa* is more closely related to *F. latifolia*, *F. uhdei*, and *F. americana* than to the Pennsylvanica Group. Although the abaxial epidermis of *F. papillosa* and *F. americana* are radically different in morphology, it seems reasonable to speculate that they may be homologous, in the sense that the same alleles underlie the shifts away from the "ground-plan" epidermis characteristic of the rest of the genus.

Fraxinus uhdei and *Fraxinus latifolia*. Results of the three molecular studies consistently indicate that these two species are not members of the Pennsylvanica Group in the concept here. Relationships of these two species with the Americana Group and *F. papillosa* are not unambiguously resolved.

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